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(54) ADMINISTRATION ORALE D'ANTICORPS A BASE DE JAUNE D'OEUF DE POULE POUR LE TRAITEMENT DE MALADIES

(54) ORAL ADMINISTRATION OF CHICKEN YOLK ANTIBODIES TO TREAT DISEASE

(57) Globalement, l'invention concerne l'utilisation de préparations d'anticorps à base d'oeuf pour le traitement de maladies touchant l'organisme entier, chez l'homme et chez d'autres mammifères. On obtient d'abord les anticorps IgY à partir d'oeufs d'une pondeuse domestique immunisée activement contre un ou plusieurs des organismes pathogènes considérés, par injection d'un immunogène contenant des déterminants immunogènes propres au déclenchement des anticorps. On administre ensuite oralement ces anticorps à un mammifère souffrant d'une maladie infectieuse touchant l'organisme, laquelle est provoquée ou accentuée par le ou lesdits organismes pathogènes. Il en résulte une immunisation passive pour les sujets à système immunitaire défaillant ou naïf. Il n'est pas nécessaire de séparer les anticorps du jaune d'oeuf, si bien que le traitement et l'administration sont pratiques et peu onéreux. Les anticorps à base de jaune d'oeuf provenant de poules immunisées contre des antigènes spécifiques sont efficaces pour lutter contre des agents toxiques de type viral, bactérien, fongique ou protozoaire et contre les toxines, les enzymes, les médiateurs inflammatoires, les prostaglandines, les leucotriènes, les thromboxines et autres molécules messagers, les sarcomes ou les carcinomes, non seulement dans l'intestin, mais encore dans les tissus éloignés de l'intestin. Le déterminant immunogène peut renfermer uniquement une partie spécifique de l'organisme pathogène, par exemple la boucle ou l'enveloppe d'un virus ou la fimbria d'une bactérie ciliée. L'efficacité du procédé est avérée dans le traitement du SIDA chez l'homme, le choc septique à médiation par facteur de nécrose tumorale (TNF) chez la souris, et dans l'abaissement du nombre des cellules somatiques chez les bovins laitiers.

(57) Broadly, the present invention is directed to the use of egg antibody preparations in the treatment of systemic disease in human and non-human mammals. IgY antibodies are first obtained from the egg of a domestic fowl hen which has been actively immunized against said one or more pathogenic organisms by injection with an immunogen containing immunogenic determinants specific to elicit such antibodies. The antibodies are then administered orally to a mammal suffering from an infectious systemic disease caused or exacerbated by such pathogenic organism or organisms. This invention, thus, is capable of providing passive immunity to patients with failing immunity or that are immunologically naive. It is unnecessary to separate the antibodies from the egg yolk, so processing and administration are convenient and inexpensive. Antibody produced from egg yolks of hens immunized against specific antigens are effective in controlling noxious agents, whether viral, bacterial, fungal, protozoal, toxins, enzymes, inflammatory mediators, prostaglandins, leukotrienes, thromboxines and other messenger molecules, sarcomas or carcinomas, not only within the bowel but also in tissues remote thereto. The immunogenic determinant may comprise only a specific portion of the pathogenic organism, e.g., the loop or coat of a virus or the fimbria of a piliated bacterium. The method of this invention has been shown to be efficacious in the treatment of AIDS in human beings, TNF mediated septic shock in mice, and in lowering somatic cell count in dairy cattle.

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(54) Title: ORAL ADMINISTRATION OF CHICKEN YOLK ANTIBODIES TO TREAT DISEASE

(57) Abstract

Broadly, the present invention is directed to the use of egg antibody preparations in the treatment of systemic disease in human and non–human mammals. IgY antibodies are first obtained from the egg of a domestic fowl hen which has been actively immunized against said one or more pathogenic organisms by injection with an immunogen containing immunogenic determinants specific to elicit such antibodies. The antibodies are then administered orally to a mammal suffering from an infectious systemic disease caused or exacerbated by such pathogenic organism or organisms. This invention, thus, is capable of providing passive immunity to patients with failing immunity or that are immunologically naive. It is unnecessary to separate the antibodies from the egg yolk, so processing and administration are convenient and inexpensive. Antibody produced from egg yolks of hens immunized against specific antigens are effective in controlling noxious agents, whether viral, bacterial, fungal, protozoal, toxins, enzymes, inflammatory mediators, prostaglandins, leukotrienes, thromboxines and other messenger molecules, sarcomas or carcinomas, not only within the bowel but also in tissues remote thereto. The immunogenic determinant may comprise only a specific portion of the pathogenic organism, e.g., the loop or coat of a virus or the fimbria of a piliated bacterium. The method of this invention has been shown to be efficacious in the treatment of AIDS in human beings, TNF mediated septic shock in mice, and in lowering somatic cell count in dairy cattle.

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ORAL ADMINISTRATION OF CHICKEN YOLK ANTIBODIES TO TREAT DISEASE

Cross-Reference to Related Applications

This application is a continuation-in-part of application serial no. 08/369,310, filed January 6, 1995, now U.S. Pat. No. ________, the disclosure of which is expressly incorporated herein by reference.

Background of the Invention

The present invention relates to the preparation of egg antibody products which may be used to neutralize a wide variety of systemic pathogens in both human and non-human species, including those with failing, naive or compromised immune systems. Its most immediate application is believed to be in the support of patients with acquired immunodeficiency syndrome (AIDS), now widely regarded as the most devastating disease in the history of humankind.

The spread of AIDS is increasing at a rapid rate. The first known death due to AIDS was in 1980. From July 1, 1992 to July 1, 1993, there were 194,500 official deaths due to AIDS, though the government says the actual number is probably at least 10% higher since not all cases are reported. There were 375,000 new cases of AIDS diagnosed between July 1, 1992 and January 1, 1993. At this time there are 315,000 patients reported to have AIDS. There is a more than 60% expected death rate.

There was $150,000.00 - $200,000.00 spent last year alone to supply health care for each victim of the AIDS virus. This value does not include the money that is currently being spent to take care of those who are still alive. Since Health and Life Insurance companies will not insure any person who is HIV positive, the above mentioned costs eventually come from the US taxpayer. This means that last year $38,900,000,000 was spent by the US taxpayers for AIDS patients. Every 13 minutes another person becomes HIV+ in the United States, which means eventually he/she will come down with AIDS. Since there is no CURE for AIDS, this means not only that the person who has AIDS gets a death warrant but also the US taxpayer is handed an additional bill every 13 minutes.

The AIDS infection is not as prevalent in the US as it is in countries in Africa or the Caribbean. AIDS is slowly spreading to all countries in the world. There were only 223 deaths from AIDS in Japan from July 1, 1992 until July 1, 1993. In the United States, one out of every 100 males and 1 out of every 800 females are currently HIV positive. It is no longer a "homosexual" disease. Today the fastest growing segment of the population is children born to HIV+ women who did not know that they carried the virus.

The immunocompromised patient may be defined as an individual who is at increased risk for infection, often to a life-threatening extent. In addition to AIDS, patients with host immune defense dysfunction most commonly observed in clinical practice include those with cancer, myeloproliferative diseases, burns, diabetes, severe trauma, or those undergoing immunosuppressive drug therapy or irradiation. The immature or naive immune system of neonates makes those with medical problems a special subset of immunocompromised patients. At the other end of the age spectrum, evidence exists to suggest that immunity declines with aging.

The gastrointestinal tract is centrally involved in the infectious status of the immunocompromised patient. Enteric infections are common and can be accompanied by debilitating diarrhea and nutrient malabsorption. Enteric organisms are further implicated as a source of bacteremias and sepsis in immunocompromised patients. It is estimated that in the US alone more than 130,000 deaths last year are associated with bacteremia. A large proportion of these resulted from microorganisms that normally reside in the gut. Even organisms that normally are considered nonpathogenic may establish local infections of secondary bacteremias. Disabling gastrointestinal symptoms are prominent both in patients with established AIDS and in patients with earlier stages of human immunodeficiency virus (HIV) infection.

Patients who are HIV-positive do not die from the virus *per se*. Rather, they eventually die from the effect of the virus on the immune system. Recurrent and progressive opportunistic infections set into motion a spiral that ultimately leads to the demise of the patient. Central to this downward clinical course is the failure of gut immunity as well as systemic immunity. Gastrointestinal organisms and inflammatory mediators normally are not pathogenic because they are neutralized by an individual's defense system; they only cause disease when these natural defenses fail. When gastrointestinal organisms overcome innate and specific immunity, they and associated inflammatory mediators cause local disease. These factors translocate across the intestinal lining to cause systemic disease. When patients die of advanced AIDS in a malnourished condition, the opportunistic infections from which they suffer are not diseases they "caught" but rather diseases from potentially pathogenic organisms they have carried in their gastrointestinal tracts for most of their lives and can no longer defend against.

The intact immune system consists of two lines of defense which keep potentially pathogenic gastrointestinal organisms from binding to the intestinal lining. First, there are non-specific barriers referred to as the innate immune system. These include stomach acid to destroy swallowed organisms, fiber to enmesh and sweep pathogenic organisms or chemicals off the intestinal surface, and a coat of "friendly" bacteria called the anaerobic paste. These non-specific barriers combine to prevent opportunistic organisms from achieving physical contact with the mucosa, or gut

lining. Second, immunity is a lymphocyte-dependent system whereby potential disease-causing organisms or substances present in an individual's intestine are "sampled" at thousands of sites throughout the small intestine known as Peyer's patches. These patches are collections of lymphocytes that identify proteins on the surface of organisms. The most effective antibodies are those produced against adhesians also known as lectans. Adhesians are the binding sites on organisms which adhere to susceptible receptor sites on tissue, thus becoming infected. A receptor is usually a glycoprotein but can be a glycolipid or a peptide called integrin. Lectans are a class of proteins on the infecting organism that can combine with these receptors rapidly, selectively and reversibly. T-lymphocytes within the Peyer's patches pass anti-adhesian information to B-lymphocytes. The B-lymphocytes, in turn, pass into the circulating blood where they become plasma cells. Plasma cells travel throughout the bloodstream and concentrate in areas where they produce specific antibody to coat moist surfaces and to prevent adhesion by the offending organisms. If a pathogenic organism cannot adhere to the tissue surface, it cannot proliferate and cause infection, circulatory antibodies perform this function in cells remote to the intestines.

If the aforedescribed system fails and an organism adheres to the tissue surface, mediators of inflammation, *e.g.*, tumor necrosis factor (TNF), interleukins 1, 6, and 8 (IL-1, -6, -8), *etc.*, are released. These mediators then initiate the production of such other mediators as prostaglandins, leukotrienes, and thromboxines. The resultant "cascade" is associated with the metabolic and physiologic changes of illness. An incompetent intestine allows translocation of organisms and disease-associated factors, such as inflammatory mediators and endotoxins, from the intestinal lumen into the systemic circulation to cause disease at sites remote to the intestinal lumen.

In certain of the aforementioned categories of immune-suppressed individuals, antibody production can return to normal if the patient does not succumb to the primary disease or opportunistic infections. With HIV infection, however, recovery of immune-competence cannot occur. The reason for this is that a favored site for HIV virus concentration is a layer of the intestinal lining called the lamina propria. The lamina propria comprises the layer just below the mucosa, or surface layer, and is the location where plasma cells concentrate to produce antibody to neutralize the lectans of potential pathogens. This antibody called secretory IgA is secreted onto the surface of the intestinal lining to bind organism adhesions, thus preventing attachment.

Plasma cells are destroyed in HIV infection either directly or as a result of destruction of lymphocytes and pathways needed for their production. Although the HIV virus cannot be eradicated, the negative effects of reduced immunity to opportunistic infections can be controlled to some extent. Infection by these opportunistic organisms stimulates the production of inflammatory mediators, as described *supra*. Principal among them is TNF. In addition to being a major stimulator

of metabolic and physiologic changes associated with illness, TNF turns on replication of the HIV virus. Thus, there exists a symbiotic relationship which results in progression of the HIV-infected patient toward AIDS.

TNF has also been identified as an important factor in causing deterioration of the brain and nervous system, pulmonary function, loss of appetite and further deterioration of the intestine leading to further opportunistic organism adhesion, diarrhea, and malabsorption. The ensuing malnutrition itself is known to depress production of antibody and lymphocyte count. Also, the lymphocyte count drops acutely in response to any infection, particularly in response to TNF. In addition to the destruction of lymphocytes by the virus, the systemic effect of an increasing rate of recurrent infections by opportunistic organisms will further suppress the lymphocyte count. It is the gut's involvement in the inflammatory process that causes the patient to lose his or her appetite and develop malabsorption, leading to malnutrition. Thus, the patient suffers from a triple indemnity, *viz.*, suppressed lymphocyte count from the virus, leading to a suppressed lymphocyte count from infection, which is then potentiated by malnutrition. This is the mechanism whereby HIV-infected patients develop AIDS, waste, and die. The virus itself does not cause the wasted appearance, nor is the individual's body mass being consumed by the virus. Rather, the wasted appearance is caused by the above chain of events leading to chronic infection, malnutrition and death.

A wide range of infectious pathogens is encountered both in patients with established AIDS and in patients in the earlier stages of HIV infection. Viruses include cytomegalovirus (CMV), herpes simplex virus (HSV), and papovavirus, of which CMV is by far the most devastating. CMV infection may simultaneously or sequentially involve the entire gastrointestinal tract, and is extremely invasive and serious, producing systemic and localized symptoms of dysphagia, odynophagia, vomiting, abdominal pain, nausea, diarrhea with or without blood and mucus, episodes of megacolon and localized peritonitis, biliary obstruction, dementia and blindness.

Bacterial pathogens include the various forms of *TB*, *Mycobacterium avium intracellulare* (MAI), *E. coli*, *Hemophilus*, *N. gonorrhoeae*, *Salmonella typhimurium*, *Campylobacter jejuni*, *Shigella flexneri*, and cholera. Patients with AIDS are especially susceptible to infection by *S. typhimurium*, which may result in life-threatening diarrhea and recurrent systemic bacteremia resistant to antibacterial therapy. MAI is the most common cause of disseminated bacterial infection in patients with AIDS. In normal or non-immunocompromised hosts, disease due to this organism is rare and clinical manifestations, if any, are primarily pulmonary. In AIDS patients, MAI typically causes a widespread infection with involvement of the bone marrow, spleen, lungs, lymph nodes, intestinal tract, brain, and adrenal glands.

Protozoa include *Isospora, Pneumocystis carinii, Toxoplasma, Entamoeba histolytica, Giardia lamblia,* and coccidia. Symptoms of *Paracytoxis* infection in immunocompetent individuals are usually of short duration, with intestinal involvement resulting in moderate to severe diarrhea and possible weight loss. In immunocompromised individuals, however, infections can become persistent and life-threatening. In a small percentage of AIDS patients, *parasites* are invasive and can produce a syndrome of overwhelming chronic and large volume diarrhea, abdominal pain, hypovolemia, electrolyte disturbances, and nutritional deficiency.

Fungi include *Candida albicans*, and *Cryptococcus, Histoplasma*. Persistent oral candidiasis is seen in a large percentage of AIDS patients and is usually thought to herald the onset of "end stage" AIDS as it invades progressing to fungal septicemia and death.

Neutralizing antibody against a variety of pathogens, *e.g.*, rotavirus, cholera, enterotoxigenic *E. coli*, *Streptococcus mutans, Salmonella, N. gonorrhea*, HIV, TNF, IL-1, IL-6, endotoxin, spider and snake venom, *etc.*, have been produced in various ways and reported in the scientific literature. Methods of production include genetically engineered bacteria, monoclonal hybridomas, and hyperimmunization of chickens and cows. Such antibodies have been shown to neutralize the targeted pathogens *in vitro* and *in vivo* in the intestine at the pathogen/mucosa interface.

Recent publications have shown that in both animal species and humans orally-administered antibodies may contribute to the therapy of infectious intestinal diseases. There have been problems with antibodies in the past. Problems included the following:

a. Monoclonal antibodies work at first, but then the organisms mutate and the monoclonal antibodies cease to work.
b. Monoclonal antibodies are destroyed by the intestines.
c. Polyclonal antibodies from hyperimmunized bovines tend to be highly variable. Some cows produce good viable antibodies, while others produce very poorly. Since each cow produces a large volume of hyperimmune whey, there is little or no mixing of samples from multiple donor cows.

Using the inventive procedure, polyclonal chicken egg antibodies prove to be quite successful and comprise the bulk of this patent. Some of the benefits of immunizing hens are as follows:

a. Polyclonal antibodies (the type produced by hens) are not destroyed by the upper intestines.
b. Since hyperimmunized hens produce polyclonal antibodies, there are many more sites for adhering to the disease-causing agent and the antibodies will

perform better through multiple mutations of a disease.

c. Each hen produces a relatively small volume of hyperimmune yolk. The product of multiple hens is blended to give a more uniform product.

Antibody from hyperimmunized hens have not been used in the past because there were concerns that the antibodies would not work across species. Experiments conducted in connection with the present invention have disproved this fear.

Several recent studies have demonstrated reduced morbidity after the administration of immunoglobulins derived from bovine milk, chicken egg, and human serum to passively protect individuals from rotavirus, parasites, enterotoxigenic diarrheal diseases caused by *E. coli*, neonatal necrotizing enterocolitis and streptococcus mutans.

In order to be effective against enteropathogenic germs, specific antibodies must reach their target site immunologically active in order to prevent the germs' adhesion, reproduction, and cell inflammatory mediator release. It has never been known with certainty just how far orally administered egg antibodies can resist the gastric acid barrier to protect infants and young domestic animals from infectious intestinal diseases. Some of the reports used anti-digestion medicators to protect the AB. Nor has it been shown or even suggested that antibody produced in a hyperimmunized yolk model could be targeted to favorably interfere with specific pathogens or substances in a site remote to the intestine when that antibody had been administered by the oral route. There exists an urgent need for a convenient yet effective method of antibody administration in the treatment of HIV-positive and other immunocompromised patients, which is not a medicine, has no known side effects and does not alter the immune system. The antibody acts like a chelating agent--searching the body for a pathogen to neutralize. There would also be ready acceptance of such a development in the veterinary and human medical fields. For instance, if antibodies could be delivered orally for systemic delivery to such remote locations as the mammary gland, where they could chelate mastitis-causing pathogens, it could revolutionize the treatment of this disease in dairy cattle. Although lacking the socioeconomic importance of such human diseases as AIDS, mastitis is an extremely important veterinary disease. In 1992, mastitis infections cost the American Dairy Association members $200,000,000. High somatic cell counts in cows result in the loss of approximately 200,000 lbs. of milk per cow per year at a financial cost of approximately $1,000 per cow. There are 9,750,000 cows in the US. Only 10% of the dairy cows in the US attain the highest ranking on somatic cell count (below 100,000). A dairyman receives $.50/100 wt of milk more when they get the highest ranking on SCC. The environment (weather) can have an effect on SCC. One important example is the floods in the Upper Midwest this past summer. In Minnesota, where this research was completed, 20 out of 26 dairies did not

pass the SCC for 2 testing periods in a row (June and July). The SCC are not monitored in August. If they had been monitored, it is highly probably that the results would have continued dropping due to the quality of the grazing areas. If the SCC value was high for 3 months in a row it would have been an automatic revocation of the sale of that milk for ANY purpose. The financial losses would have been enormous. Normally the month of August is the best month of the year for milk production, therefore the ADIP does not monitor SCC.

Further information can be found by reading Ungar *et al.*, "Cessation of Cryptosporidium-Associated Diarrhea in an Acquired Immunodeficiency Syndrome Patient After Treatment with Hyperimmune Bovine Colostrum," *Gastroenterology*, 1990, 98:486-489; Tzipori *et al.*, "Remission of Diarrhea due to Cryptosporidiosis in an Immunodeficient Child Treated with Hyperimmune Bovine Colostrum," *British Medical Journal*, vol. 293, 15 Nov. 1986, pp. 1276-1277; Yolken *et al.*, "Immunoglobulins and Other Modalities for the Prevention and Treatment of Enteric Viral Infections," *J. Clin. Immunol.*, vol. 10, no. 6, Nov. 1990, pp. 80S-86S; Bernhisel-Broadbent *et al.*, "Allergenicity of Orally Administered Immunoglobulin Preparations in Food-Allergic Children, *Pediatrics*, vol. 87, no. 2, Feb. 1991, pp. 208-214; Zanetti *et al.*, "Use of Immunoglobulins in Prevention and Treatment of Infection in Critically Ill Patients: Review and Critique," *Reviews of Infectious Diseases*, 1991, 13: 985-992; Kühlmann *et al.*, "Chicken Egg Antibodies for Prophylaxis and Therapy of Infectious Intestinal Diseases: Immunization and Antibody Determination," *J. Vet. Med.*, 1988, 35: 610-616; Wiedemann *et al.*, "Chicken Egg Antibodies for Prophylaxis and Therapy of Infectious Intestinal Diseases: *In Vivo* Tenacity Test in Piglets with Artificial Jejunal Fistula," *J. Vet. Med.*, 1990, 37: 163-172; Weidemann, *et al.*, "Chicken Egg Antibodies for Prophylaxis and Therapy of Infectious Intestinal Diseases: *In Vivo* Studies on Protective Effects against *Escherichia Coli* Diarrhea in Pigs" *J. Vet. Med.*, 1991, 38: 283-291; Schmidt *et al.*, "Chicken Egg Antibodies for Prophylaxis and Therapy of Infectious Intestinal Diseases: *In Vitro* Studies on Gastric and Enteric Digestion of Egg Yolk Antibodies Specific against Pathogenic *Escherichia Coli* Strains," *J. Vet. Med.*, 1989, 36: 619-628; Lösch, *et al.*, "The Chicken Egg, an Antibody Source," *J. Vet. Med*, 1986, 33: 609-619; Jüngling *et al.*, "Chicken Egg Antibodies for Prophylaxis and Therapy of Infectious Intestinal Diseases: *In Vitro* Studies on Protective Effects against Adhesion of Enterotoxogenic *Escherichia coli* to Isolated Enterocytes," *J. Vet. Med.*, 1991, 38: 373-381; Ikemori *et al.*, "Protection of Neonatal Calves Against Fatal Enteric Colibacillosis by Administration of Egg Yolk Powder from Hens Immunized with K99-Piliated Enterotoxigenic *Escherichia coli*," *Am. J. Vet. Res.*, vol. 53, no. 11, Nov. 1992; and Yokoyama *et al.*, "Passive Protective Effect of Chicken Egg Yolk Immunoglobulins against Experimental Enterotoxigenic *Escherichia coli* Infection in Neonatal Piglets," *Infect. Immun.*, 1992,

60(3): 998-1007.

A useful overview of background information can be found by reading: Sharon et al, *"Carbohydrates in Cell Recognition"*. Sci. Amer. Jan 1993.

The approach taken herein has been validated in U.S. Pat. No. ________, cited above, for lowering somatic cell count in the milk of lactating ruminants.

Broad Statement of the Invention

Broadly, the present invention is directed to the use of egg antibody preparations in the treatment of systemic disease in human and non-human mammals. IgY antibodies are first obtained from the egg of a domestic fowl hen which has been actively immunized against said one or more pathogenic organisms or noxious agents by injection with an immunogen containing immunogenic determinants specific to elicit such antibodies. The procedure for injecting the hens with the immunogens was taken from Fertel *et al.*, "Formation of antibodies to prostaglandins in the Yolk of Chicken Eggs," *Biochem. Biophys. Res. Comm.*, 1981, 102: 1028-1033. The antibodies then are administered orally to a mammal suffering from or to prevent an infectious or non-infectious systemic disease caused or exacerbated by such pathogenic organism or organisms or noxious agents. This invention is thus capable of providing systemic passive immunity. Concentration of the antibody can be done, but it is unnecessary to separate the antibodies from the egg yolk, so processing and administration are convenient and inexpensive.

Antibody produced from egg yolks of hens immunized against specific antigens are effective in controlling noxious agents, whether viral, bacterial, fungal, protozoal, toxins, inflammatory mediators, prostaglandins, leukotrienes, thromboxines, sarcomas or carcinomas, not only within the bowel but also in tissues remote thereto. The immunogenic determinant may comprise only a specific portion of the pathogenic organism, *e.g.*, the loop or coat of a virus or the fimbria of a piliated bacterium. The method of this invention has been shown to be efficacious in the treatment of septic shock in mice, and in lowering somatic cell count in dairy cattle. Thus, the discovery disclosed herein shows that an egg antibody raised against any of the pathogenic organisms or molecules discussed in the Background can be effective in controlling this disease in tissue remote from the intestine, *i.e.*, systemic disease.

Detailed Description of the Invention

The mucous membranes, including the gastrointestinal tract, share in immunoglobulin secretion, a function distinct from other lymphoid tissues in the body. Secretory immunoglobulins prevent the adherence of pathogens and other mucosae or most surfaces of the body and, thus, prevent their ability to cause disease. Proper

function of the secretory immune system requires the coordinated activity of mucosae cells, antigen-processing cells, several classes of T cells, and plasma cells. A deficiency of secretory IgA, the major secretory immunoglobulin, may be associated with recurrent intestinal, pulmonary, sinus or other systemic infections. Secretory IgA is produced by plasma cells found in the lamina propria and is believed to play an important role in gut immunity. These plasma cells are transformed programmed B cell lymphocytes, the programming of which depends on signals from the T cell in the lymphoid tissue of the intestine which is concentrated in areas called Peyer's patches.

Critical to intestinal immunity is the production of antibody which prevents gut surface infection. T-cell lymphocytes found in Peyer's patches process a pathogen's surface proteins, which are responsible for its binding to the intestinal mucosa. If there is no adhesion, there can be no disease. Thus, an antibody against these surface proteins can prevent binding and disease. Intestinal opportunistic organisms are not killed by such an antibody, but the antibody neutralizes the organisms preventing binding to the mucosa. While several factors interact to prevent potential pathogens from adhering to the mucosa, secretory IgA is specific and the cornerstone of all the defense mechanisms.

The primary defect in AIDS is felt to be the destruction of specific subsets of helper T lymphocytes, due to infection by the retrovirus HIV. The immunological derangement is widespread, including functional abnormalities of T cells, B cells, antigen-processing cells, and macrophages. The development of mucous membrane infections such as CMV and MAI leading to invasion and disseminated disease or septocemia, implies that secretory immunity is impaired in patients with AIDS.

A newborn mammal is a good example of an immunologically incompetent individual and the benefits of passive immunity by the oral ingestion of antibody. Despite the fact that a newborn's intestinal immunity is not capable of adult immunologic behavior, the neonate does not ordinarily succumb to organisms ingested from its environment that are potentially disease-causing and lethal. This is because the neonate's mother has already developed immunity against the organisms in her environment. She passes that immunity on to her offspring in the form of antibodies, by delivering high concentrations of antibody via her mammary gland into the mouth and intestine of the suckling neonate. For example, it has been shown in the cow, guinea pig, and human that large concentrations of plasma cells are found in the mammary gland just prior to parturition. The first suckling of the newborn produces colostrum, which is not milky but rather straw-colored and somewhat resembling honey in appearance. Colostrum is essentially pure antibody.

Thus, bovine secretory antibody provides passive immunity to an immunologically naive calf that renders the calf safe from all barnyard parasites, bacteria and fungi which the mother cow has ingested and has in her intestinal

environment. It has been shown that, for the first 12 hours, the colostrum ingested by a calf passes directly into the blood as if it were given intravenously. Chickens also produce antibody to protect the baby chick from the barnyard's organism environment. In avian species, the progeny acquire passive immunity by the absorption of maternal antibodies transferred to the egg, particularly the yolk. Thus, baby chicks are born immunologically naive in terms of their own antibody production but are not harmed by barnyard pathogens because their mother has actively provided antibodies against all such pathogens in the yolk.

It is well known that antibody produced in one species can be used to neutralize the effects of the corresponding antigen in other species. Passive immunization thus also occurs when an individual from one species receives immune protection from antibodies produced in an individual of another species. When the antigen used in immunization of the hen is a bacterium which causes intestinal infectious diseases such as colibacillosis in calves or piglets, the antibody-containing yolk obtained from an egg of the immunized hen in the aforedescribed manner has an activity against the antigen and thus is effective in protection of calves or piglets from attack by the same bacterium used in the immunization. For example, laying hens may be immunized with a vaccine for pregnant sows in order to obtain high amounts of specific antibodies against porcine enteropathogenic *E. coli* strains. The resultant antibody-containing eggs are then mixed with milk replacer and fed to piglets to treat intestinal colibacillosis.

Since most enteropathic organisms that cause the progressive deterioration of the AIDS patient can be found in the "barnyard" environment, it would appear to be advantageous to have patients with failing gut immunity consume colostrum or egg yolks containing antibodies to those organisms. Both cows and chickens can be "trained" to produce the antibodies that are needed to protect an individual with a failing immune system. In addition, bovine and avian antibodies have the important advantage of being somewhat protected from digestion unlike, for example, monoclonal antibodies or human serum antibodies.

While colostrum and egg yolks are both effective in providing immunity, chickens possess certain advantages over cows which will be readily apparent to those familiar with both species. Cows are expensive and produce a calf, and thus colostrum, only once a year. The higher concentrations of colostrum are only available for 1-2 days and must be refrigerated. In contrast, a hen lays eggs 7 out of 10 days. Eggs can be stored even at room temperature for several weeks. Once a hen has "learned" to produce antibodies against a specific antigen, it will do so for its entire life, which can span 10 years. Furthermore, the average egg contains 15 ml of yolk having 8 mg/ml of IgG, also referred to as IgY, or "yolk immunoglobulin." This makes the chicken a much more efficient antibody producer than the cow. Chickens produce approximately 20 times more antibody per kg body weight than a cow does in

colostrum. In addition to chickens, other domesticated fowl may also serve as sources of eggs, *e.g.*, turkeys, ducks, geese, and the like.

The laying hen transfers all antibody isotopes found in the chicken to the egg, *i.e.*, IgY, IgM, an IgA antibodies. The yolk contains only IgY while IgM and IgA are found only in the white. The chicken's serum IgY antibody level is reflected in the egg yolk shortly after a single administration of antigen (about one week). Egg yolk contains 3-25 mg IgY/ml. Depending on its weight, therefore, each egg could provide 40-500 mg IgY.

The advantages of egg yolk antibodies are numerous. Chicken antibodies do not react with mammalian complement, Fc receptors, protein A or protein G. Yolk antibodies show great acid and heat resistance. Extraction of yolk antibodies can be performed even on a large scale without costly investment. Concentrating the antibody from egg yolk is a relatively straightforward process. The antibody is not harmed by pasteurization. The FDA regards egg antibody as a food rather than a drug and has granted GRAS (generally accepted as safe) status thereto. Approval by the FDA for human use of egg antibody in the AIDS or other diseases is relatively unencumbered.

The net effect of treating the HIV patient with egg antibodies is not to prevent the virus from replicating, binding and translocating into the patient from the intestine, but rather to neutralize the virus in areas remote to the intestines. Regardless of the manner in which a patient becomes infected with the HIV virus, eventually the highest concentrations are found in the intestinal lamina propria. The virus incubates in this environment requiring TNF to initiate and speed replication. Thus, production of antibody to neutralize the HIV virus itself and the inflammatory mediator TNF is as important in slowing the disease as is neutralization of opportunistic organisms.

Another approach might be described as "personalized" avian surrogate secretory IgA. It is axiomatic that the stool of an HIV-positive patient contains the organisms from which that patient is likely to die. Therefore, in addition to producing antibody to neutralize the HIV virus and TNF, the stool of a patient could be collected, subcultured, combined, and sterilized and used to raise antibody in a colony of chickens dedicated to that individual. Experiments have been performed comparing the antibody levels and effectiveness in fresh eggs, pasteurized egg yolk and dried egg yolk that indicate that the product is stable in all of these environments. The yolk would then contain antibody against everything in the intestine that would cross into the blood stream and make that individual "ill" as the immune system deteriorated. Just as a mother chicken passes immunity to its chick, this colony of chickens would pass the same immunity to the HIV-positive patient. Alternatively, the stool of a patient positive for HIV could be cultured. As specific organisms were identified, a "cocktail" of avian antibody raised against each organism would be blended to neutralize their ability to create systemic disease.

The fact that the egg antibody preparations disclosed herein have been found to be exceptionally useful with respect to the special needs of HIV patients should in no way be construed as limiting the invention to this particular class of patient. Burn patients, organ transplant patients, patients in intensive care units, and patients with autoimmune diseases provide further examples of individuals that might benefit from the present invention. Enteric infections which translocate systematically are major causes of problems in these patient populations as well. Also, viral infections of the gastrointestinal tract with systemic manifestations constitute a major health problem during the first years of a child's life, and prolonged infections occur in patients rendered immunocompromised by congenital or acquired immunodeficiency states. In children with impaired T-cell function, for example, rotavirus infection is an especially serious problem. Any molecule can be used to produce antibody that can be fed orally to down-regulate the disease. Such molecules have been found in transplant regions, multiple sclerosis, viral replication, cancer, cytokine production, *etc.* It will be readily apparent that the present invention possesses utility in these areas as well.

Veterinary applications of the present invention include, surprisingly, the treatment of mastitis in dairy cattle, as disclosed in U.S. Pat. No. ______, cited above.

It is our opinion that the hyperimmune yolk antibody works in the following manner, Note Fig. 1, 2. The IgY molecule has a "Y" shape. The tail of the Y has three constant amine acid configurations or domains on it that identify the chicken origin. We will call these C1, C2 and C3. The higher of the "v" portion of the Y contains active particles of less than 100 amino acid units. This is called the hypervariable area (VlVh) and is the actual portion of the antibody that attaches to the noxious agent. The units that make up the Y molecule are linked by disulfide bonds. These bonds are attacked in the intestines by papain and pepsin. Papain digests one joint of the v portion of the Y releasing the VlVh which is absorbed as a non-antigen peptide into the blood. The "tail" of the Y molecule is sent through the digestive as any protein. The VlVh is now a negatively charged. The negative charged hypervariable segment peptide binds to positively charged areas on human globulin protein. The human globulin carries the antibodies to the target organ. When the specific amino acid sequence portion of the v finds its complement, it locks into the receptor of the noxious agent and neutralizes it. If attached to the patient's globulin, the complex may activate a complement. The complement causes monocytes to adhere to the compound and phagocytosis occurs. Alternatively, the large peptides of the V1Vh circulate carried by other proteins, such as albumum. They bind to the target pathogen they were formed against and compromise adhesion to body tissues. If there is no adhesion, there is no disease and pathogen is cleared from the body.

The adhesian to the host is an important factor of primary virulence. A variety of adhesive structures, broadly referred to as adhesions or lectans, on the surfaces of

microorganisms serve to bind them to complementary adhesive structures on the surfaces of host cells known as receptors. This is very specific and similar to a lock and key. Some pathogenic organisms have also developed proteinaceous surface structures, such as the fimbriae, or pili, like *E. coli* and *c. albicous*. Such structures play a role in the interaction of the organism with the glycoprotein receptor of host cells. Adhesion of the pili lectan to the host cell surfaces glycoprotein receptor or basement membranes serves as an essential first step in the pathogenesis of disease.

Antibody can be raised against certain portions of pathogenic organisms adhesans rather than the organisms in entirety. For example, it has been found in connection with the present invention that antibody raised against the specific adhesion of an enteropathogenic organism, *e.g.*, the pilus in fimbriated bacteria such as *E. coli, Cholera vibro, salmonella, gonococcus, H. flu, proteus mircobilis and actinomyces viscosus* is extremely effective in preventing disease caused by that organism. More specifically, the cell receptor is now known for the most part to be a glycoprotein or glycolipid, two types of complex carbohydrates in which sugars are linked to proteins and lipids, respectively. Several thousand such receptors have been found. The adhesan of the pathogen is now known to be a lectin once thought to be found only in plants. They are found on the surface of pathogens strategically positioned to combine with very specific carbohydrate receptors of the susceptible cell. Lectans can be identified on toxins, viruses, bacterium, fungus, cancer cells, and other molecules identified as causing disease. It has been shown that antibody could thus be effectively raised against analogous structures. Examples of such structures might include the "eoat" of CMV virus, the "gp120 " or "V_3" loop of HIV virus, *etc*. Specifically, the V_3 loop of the gp120 portion of the protein coat of the HIV virus is the site which specifically combines with the CD_4 receptor of the T-cell lymphocyte. Antibody against this lectin will neutralize its ability to bind to and infect a T-cell. This will be supplemented with anti-TNF antibody. Neutralizing TNF will suppress HIV replication in that TNF turns on the replicating gene of the HIV virus. TNF also is a mediator of metabolic and physiologic changes associated with illness in general. These effects occur throughout the body and primarily in the lamina propria of the intestine which is a site remote to the intestinal lumen. Similarly, glycoprotein B is the lectin for CMV. CMV has an affinity for nerve tissue, especially the optic nerve. Prophylaxis is thus important for all immune suppressed individuals, especially transplant patients where 25% have evidence of the disease. Candida albicans has a fimbriae adhesan. The lectin is located at the end of the structure. Candida pharyngitis or esophagitis is painful but candida septicemia is potentially lethal. It is common in immune suppressed populations and in diabetic patients. IgY raised against lectans of this pathogen will control local and systemic manifestations of this disease.

The techniques for immunization of a hen against selected antigens are well-known to those in the art. Briefly, immunization may be performed by inoculation with the antigen by any appropriate route such as subcutaneous, intraperitoneal, intramuscular, or intravenous injection, or oral administration. The preferred method of immunization is by intramuscular injection, preferably subcutaneous on the neck. Preferably a suitable adjuvant is administered in conjunction with the antigen to enhance the immunization. An adjuvant useful for this purpose is a water-in-oil emulsion adjuvant such as complete Freund's adjuvant (CFA). It has been found that the use of a suitable adjuvant is highly effective in maintaining a high antibody titer in the eggs of an immunized hen for a prolonged period, thereby making it possible to produce the desired antibody-containing substance efficiently. The dose of the antigen is determined depending on the type of the antigen and adjuvant and the administration route in such a manner that an immune status is induced in the hen without development of excessive antigen toxicity.

Usually within a few weeks following the initial immunization (inoculation), the hen becomes sensitive to the antigen, *i.e.*, immunized against the antigen. A specific antibody against the antigen is produced within the body of the hen, and an egg laid by the hen contains the specific antibody. The presence and the titer level of the specific antibody against the antigen in the hen and in eggs of the hen can be confirmed by a number of methods known to those skilled in the art of immunological tests.

After the initial immunization of the hen against the antigen, one or more boosters at an appropriate dose level may be administered in order to maintain a high antibody titer in the hen. Again in each booster administration, a suitable adjuvant may be used in conjunction with the antigen. The interval between the initial immunization and the first booster administration and between individual booster administrations depends on the specific characteristics of the antigen and is preferably at least two weeks.

After it is confirmed that an adequate titer of the desired specific antibody is present in an egg laid by the immunized hen, an egg laid by the hen is collected and, if necessary, stored until use. Conveniently, a plurality of eggs laid by one or more hens which have been immunized against the same antibody are collected and processed together to produce the desired substance which contains the antibody.

Since most antibodies are contained in the yolk of an egg, the yolk is usually separated from the collected egg or eggs for use in the production of the desired antibody. These antibodies then can be digested in vitro and the peptides of the hypervariable segments used to neutralize targeted molecules.

Other forms which the antibody product might assume are as follows:

a. Suppositories for use in inflammatory proctitis, *i.e.* anti gc or anti TNF for

systemic treatment of gonorrheal proctitis and for Crohn's proctitis providing higher concentrations in tissue in the affected area.

b. Creams which might be used for other known sexually transmitted diseases or antibodies for treatment of tissues around burns or large surface ulcers.

c. Inhalants which might protect mast cells in the tissues of the lung from being exposed to toxic organisms, anti TB or PCP (pneumocistis pneumonia) which can replace expensive antibiotics which have side effects.

d. Soaps or other cleaning agents which might disinfect deeper tissues of wounds of patients contaminated with organisms, such as, for example, anti staph, anti E. coli and anti C. deficeal, MRSA (methicillin resistant staph aureus major help problem when patients are moved between different health residencies).

e. Mouth washes which could be used by dental patients and practitioners to protect against transmission of HIV or help treat gum tissues affected by periodontal dieases.

f. Wipes or other disposable products for clean up during surgical operations dressing changes, anti staph or anti MRSA.

g. Adhered to filters as an immediate test when blood is collected for later use; anti HIV, anti Hepatitis. This could also form filters to selectively separate certain cells from the blood. For example, one could be made to pull out T cells; if we made an anti CD4 antibody we could then give healthy CD4 cells to transplant patients. This would be good for leukemics or other patients who have been chemically devoid of T cells by immunosupressive therapy. This will cut hospitalization and costs.

h. Tablets taken on trips to protect patients against travelers' diarrhea and diseases causing suppressive diseases, such as cholera and salmonella.

i. As part of a nutritional formula given to patients in ICU or during recuperation of intestinal surgery as protection from systemic sporead from the digestive tract during period of reduced consumption.

j. Can be highly purified non-antigenic pepetides to be given either by pill for those patients who can not take the volumes needed or IV in those patients which are not able to receive any nourishments any other way

l. Anti candida albicans to prophylaxis or treat systemic candida in immune suppressed patients (including diabetics).

The following Examples demonstrate how the present invention has been practiced, but should not be construed as limiting. In this application, all citations are expressly incorporated herein by reference.

EXAMPLE 1

Survival studies were conducted in mice challenged with LD 100% dose of LPS by intrapoeritoneal injection. Twenty animals were in each group. The animals were fed either TNF IgY (produced as described in U.S. Pat. No. ______, cited above) orally or saline control by holding the animal upright and then using a specifically designed feeding syringe with a blunt end to adminster the solution into the stomach.

Typically, most of the animals subjected to this dose of endotoxin will be dead in 24 hours and none survive 48 hours. This was the result demonstrated in the control group. However, the anti-TNF treated animals showed greater than 80% survival at 24 hours and only an 80% mortality at 48 hours.

TABLE I

HOURS POST LPS INJECTION	CONTROL MICE (% Survival)	ANTI-TNF IgY MICE (% Survival)
12	100	100
18	60	90
24	19	81
30	15	52
42	0	23
8	0	18

We claim:

1. A method for treating systemic manifestations of an infectious non-intestinal disease associated with pathogenic organisms or molecules in a mammal, said method comprising orally administering to said mammal IgY antibodies obtained from the egg of a domestic fowl hen which has been actively immunized against said pathogenic organisms by injection of the hen with an immunogen containing immunogenic determinants specific to elicit said antibodies.

2. The method of claim 1, wherein said mammal is a human being.

3. The method of claim 1, wherein said mammal is immunodeficient or immunocompromised.

4. The method of claim 2, wherein said human being is HIV-positive.

7. The method of claim 1, wherein at least one of said one or more pathogenic organisms is a virus.

8. The method of claim 7, wherein said virus is selected from the group consisting of human immunodeficiency virus, cytomegalovirus, herpes simplex virus, and papovavirus.

9. The method of claim 1, wherein at least one of said one or more pathogenic organisms is a bacterium.

10. The method of claim 9, wherein said bacterium is selected from the group consisting of *Mycobacterium avium intracellulare*, *Salmonella typhimurium*, hemophilus, heliobactera pylori, cholera, gonorrhea, e pili, s pili, and like invading organisms causing systemic disease.

11. The method of claim 1, wherein at least one of said one or more pathogenic organisms is a protozoan.

12. The method of claim 11, wherein said protozoan is selected from the group consisting of *Pneumocystis carinii*, and other invading protozoa which are prevalent in HIV patients and other immume compromised individuals.

13. The method of claim 1, wherein at least one of said one or more pathogenic organisms is an invading fungus.

14. The method of claim 13, wherein said fungus is selected from the group consisting of *Candida albicans*, and other invading fungi which are prevalent in HIV and immune suppressed patients.

15. The method of claim 1, wherein said immunogenic determinant comprises a non pili adhesian.

16. The method of claim 15, wherein said adhesian comprises the gp 120 or V_3 loop or other amino acid sequence of human immunodeficiency virus.

17. The method of claim 15, wherein said adhesian comprises the "coat" of cytomegalovirus.

18. The method of claim 1, wherein in at least one or more pathogenic organisms is a systemic inflammatory mediator.

19. The method of claim 18, wherein said mediator is one or more of a cytokine, prostaglandin, thromboxine, or a leukotriene.

20. The method of claim 18, wherein said mediator is a hormone or hormone-like molecule.

21. The method of claim 20, wherein said mediator is one or more of insulin, glucogen, epinephrine, estrogen, growth hormone, progesterone, leuteinizing hormone, testoserone, aldosteron, neuroteusen, gastrin, cholecytokines, and like hormones and their metabolites.

22. The method of claim 18, wherein said mediator is a growth factor or an anti-growth factor.

23. The method of claim 22, wherein said mediator is GSF, erythrocyte growth factor, angiogenic stimulating factor and like factors and their metabolites.

24. The method of claim 18, wherein said mediator is a cell adhesian.

25. The method of claim 18, wherein said mediator is a CD4 receptor or hepatocyte receptor for colon and breast cancer or like cell surface receptors for cancer cells.

26. The method of claim 18, wherein said mediator is an intercellular mediator or enzyme.

27. The method of claim 18, wherein said mediator is one or more of nuclear factor kB, IkB, protease, cyclooxynase, or other intracellular mediator or enzyme.

28. The method of claim 18, wherein said mediator is an extracellular enzyme or mediator.

29. The method of claim 18, wherein said mediator is one or more of collagenase, thrombin, anti-thrombin, and endotoxin.

30. The method of claim 18, wherein said mediator is a receptor site or cell responsible for rejection of transplanted organs or tissues.

31. The method of claim 18, wherein said mediator is one or more of a breast cell, lung cell, liver cell, intestine cell, kidney cell, red blood cell, or white blood cell.

32. The method of claim 18, wherein said mediator is one or more of a receptor site, cell surface responsible for tissue drainage, or the noxious molecule associated with autoimmune diseases.

33. The method of claim 18, wherein said mediator is one or more of scleredema, sclerodema, multiple sclerosis, diabetes, rheumatoid arthritis, rheumatoid heart disease, or rhemotoid kidney disease.

34. The method of claim 1, wherein said IgY antibodies are obtained from the yolk of said egg without fractionation thereof.

35. The method of claim 1, wherein the IgY antibodies are in the form of a powder obtained by stirring the yolk of said egg into an emulsion and drying the emulsion to form a powder.

36. The method of claim 1, wherein the immunization of said hen is performed by administration of the antigen in conjunction with a water-in-oil emulsion adjuvant.

37. A method for treating infectious diseases associated with pathogenic organisms in an HIV-positive or immune compromised patient, which comprises: orally administering to said patient IgY antibodies obtained from the egg of a domestic fowl hen which has been actively immunized against said pathogenic organisms by injection with fecal material from said immune suppressed patient.

38. The method of claim 37, wherein said fowl is selected from the group consisting of a chicken, a duck, a goose, and a turkey.

39. The method of claim 38, wherein said fowl is a chicken.

40. The method of claim 37, wherein said IgY antibodies are obtained from the yolk of said egg without fractionation thereof.

41. The method of claim 37, wherein the IgY antibodies are in the form of a powder obtained by stirring the yolk of said egg into an emulsion and drying the emulsion to form a powder.

42. The method of claim 37, wherein the immunization of said hen is performed by administration of the antigen in conjunction with a water-in-oil emulsion adjuvant.

43. A method for treating systemic manifestations of cancer in a mammal, said method comprising orally administering to said mammal IgY antibodies obtained from the egg of a domestic fowl hen which has been actively immunized against cancer by injection of the hen with cells of said cancer specific to elicit said mammal IgY antibodies which intact or a fragment thereof are associated with a radioactive isotope or other cellular poison to the cancer cell.

44. The method of claim 43, wherein the cancer is of the brain or nervous system, skin, gastrointestinal system, respiratory sytem, musculosketetal system, or circulatory system.

45. The method of claim 43, wherein said IgY antibody, in whole or in part, is used to carry a molecule toxic to said cancer selected from a virus, a bacterium, a fungus, or a protozoa.

46. The method of claim 1, wherein said IgY antibodies are included in one or more of a suppository, a cream, an inhalant, a cleaning agent, a mouth wash, a wipe, a filter, a tablet, or a nutritional formula.

AMENDED CLAIMS

[received by the International Bureau on 17 February 1998 (17.02.98), original claims 1, 7, 9-11, 13 and 18-33 amended; remaining claims unchanged (5 pages)]

1. A method for treating systemic manifestations of an infectious non-intestinal disease associated with a pathogenic organism or molecule in a mammal, said method comprising orally administering to said mammal IgY antibodies obtained from the egg of a domestic fowl hen which has been actively immunized against said pathogenic organisms by injection of the hen with an immunogen containing immunogenic determinants specific to elicit said antibodies.

2. The method of claim 1, wherein said mammal is a human being.

3. The method of claim 1, wherein said mammal is immunodeficient or immunocompromised.

4. The method of claim 2, wherein said human being is HIV-positive.

7. The method of claim 1, wherein said pathogenic organism is a virus.

8. The method of claim 7, wherein said virus is selected from the group consisting of human immunodeficiency virus, cytomegalovirus, herpes simplex virus, and papovavirus.

9. The method of claim 1, wherein said pathogenic organism is a bacterium.

10. The method of claim 9, wherein said bacterium is selected from the group consisting of Mycobacterium avium intracellulare, Salmonella typhimurium, hemophilus, heliobactera pylori, cholera, gonorrhea, e pili, s pili.

11. The method of claim 1, wherein said pathogenic organism is a protozoan.

12. The method of claim 11, wherein said protozoan is selected from the group consisting of Pneumocystis carinii, and other invading protozoa which are prevalent in HIV patients and other immume compromised individuals.

13. The method of claim 1, wherein said pathogenic organism is an invading fungus.

14. The method of claim 13 ,wherein said fungus is selected from the group consisting of Candida albicans, and other invading fungi which are prevalent in HIV and immune suppressed patients.

15. The method of claim 1, wherein said immunogenic determinant comprises a non pili adhesian.

16. The method of claim 15, wherein said adhesian comprises the gp 120 or V3 loop or other amino acid sequence of human immunodeficiency virus.

17. The method of claim 15, wherein said adhesian comprises the "coat" of cytomegalovirus.

18. The method of claim 1, wherein said pathogenic organism is a systemic inflammatory mediator.

19. The method of claim 18, wherein said mediator mediates one or more of a cytokine, prostaglandin, thromboxine, or a leukotriene.

20. The method of claim 18, wherein said mediator mediates a hormone.

21. The method of claim 20, wherein said mediator mediates one or more of insulin, glucogen, epinephrine, estrogen, growth hormone, progesterone, leuteinizing hormone, testosterone, aldosteron, neuroteusen, gastrin, cholecytokines, and their metabolites.

22. The method of claim 18, wherein said mediator mediates a growth factor.

23. The method of claim 22, wherein said mediator mediates GSF, erythrocyte growth factor, angiogenic stimulating factor, and their metabolites.

24. The method of claim 18, wherein said mediator mediates a cell adhesian.

25. The method of claim 18, wherein said mediator mediates a CD4 receptor or hepatocyte receptor for colon and breast cancer.

26. The method of claim 18, wherein said mediator mediates an intercellular mediator or enzyme.

27. The method of claim 18, wherein said mediator mediates one or more of nuclear factor kB, IkB, protease, cyclooxynase, or other intracellular mediator or enzyme.

28. The method of claim 18, wherein said mediator mediates an extracellular enzyme or mediator.

29. The method of claim 18, wherein said mediator mediates one or more of collagenase, thrombin, anti-thrombin, and endotoxin.

30. The method of claim 18, wherein said mediator mediates a receptor site or cell responsible for rejection of transplanted organs or tissues.

31. The method of claim 18, wherein said mediator mediates one or more of a breast cell, lung cell, liver cell, intestine cell, kidney cell, red blood cell, or white blood cell.

32. The method of claim 18, wherein said mediator mediates one or more of a receptor site or a cell surface responsible for tissue drainage.

33. The method of claim 18, wherein said mediator mediates one or more of scleredema, sclerodema, multiple sclerosis, diabetes, rheumatoid arthritis, rheumatoid heart disease, or rhemotoid kidney disease.

34. The method of claim 1, wherein said IgY antibodies are obtained from the yolk of said egg without fractionation thereof.

35. The method of claim 1, wherein the IgY antibodies are in the form of a powder obtained by stirring the yolk of said egg into an emulsion and drying the emulsion to form a powder.

36. The method of claim 1, wherein the immunization of said hen is performed by administration of the antigen in conjunction with a water-in-oil emulsion adjuvant.

37. A method for treating infectious diseases associated with pathogenic organisms in an HIV-positive or immune compromised patient, which comprises: orally administering to said patient IgY antibodies obtained from the egg of a domestic fowl hen which has been actively immunized against said pathogenic organisms by injection with fecal material from said immune suppressed patient.

38. The method of claim 37, wherein said fowl is selected from the group consisting of a chicken, a duck, a goose, and a turkey.

39. The method of claim 38, wherein said fowl is a chicken.

40. The method of claim 37, wherein said IgY antibodies are obtained from the yolk of said egg without fractionation thereof.

41. The method of claim 37, wherein the IgY antibodies are in the form of a powder obtained by stirring the yolk of said egg into an emulsion and drying the emulsion to form a powder.

42. The method of claim 37, wherein the immunization of said hen is performed by administration of the antigen in conjunction with a water-in-oil emulsion adjuvant.

43. A method for treating systemic manifestations of cancer in a mammal, said method comprising orally administering to said mammal IgY antibodies obtained from the egg of a domestic fowl hen which has been actively immunized against cancer by injection of the hen with cells of said cancer specific to elicit said mammal IgY antibodies which intact or a fragment thereof are associated with a radioactive isotope or other cellular poison to the cancer cell.

44. The method of claim 43, wherein the cancer is of the brain or nervous system, skin, gastrointestinal system, respiratory sytem, musculosketetal system, or circulatory system.

45. The method of claim 43, wherein said IgY antibody, in whole or in part, is used to carry a molecule toxic to said cancer selected from a virus, a bacterium, a fungus, or a protozoa.

AMENDED SHEET (ARTICLE 19)

46. The method of claim 1, wherein said IgY antibodies are included in one or more of a suppository, a cream, an inhalant, a cleaning agent, a mouth wash, a wipe, a filter, a tablet, or a nutritional formula.

STATEMENT UNDER ARTICLE 19

Submitted herewith are substitute pages 17-21 inclusive, wherein claims 1-46 have been revised.

Specifically, claim 1 has been revised to use the singular form of "organism" and "pathogen" as an antecedent basis for claims dependent thereon.

Claims 7, 9, 11, 13, and 18 have been amended by replacing "at least one of said one or more" with "said".

Claims 10, 20, 21, 22, 23, 25, and 32 have been revised to delete certain indefinite expressions.

Claims 19-33, inclusive, also have been amended to replace "is" with "mediates".

It is noted that none of the art cited in the international search report teach the <u>oral</u> administration of an egg antibody to a mammal to deal with <u>systemic</u> disease. "Thus, the discovery disclosed herein shows that an egg antibody raised against any of the pathogenic organisms or molecules discussed in the Background can be effective in controlling this disease in tissue remote from the intestine, *i.e.*, systemic disease." (page 8, ll. 29-32 of the application". Disease remote from the gastrointestinal tract quite unexpectedly can be treated via oral administration of an egg antibody to a mammal. This is the basis of the invention as defined in the claims and which is not even remotely found in the art.

In view of the amended claims submitted herewith, Applicant respectfully submits that the statement under Article 35(2) should be in the positive in respect of the claims being novel and expressing inventive step as required.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/17722

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/138.1, 157.1, 159.1, 164.1, 172.1, 803, 802; 530/389.1, 389.4, 389.5, 389.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, BIOSIS, EMBASE, DERWENT WPI, search terms: author name, Igy, egg antibody, heliobactera, pylori, cancer, tumor, bacteria, virus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,420,253 A (EMERY et al.) 30 May 1995, see entire document.	1-46
Y	US 4,550,019 A (POLSON) 29 October 1995, see entire document.	1-46
Y	US 5,260,057 A (CORDLE et al.) 09 November 1993, see entire document.	1-46
Y	US 4,748,018 A (STOLLE et al.) 31 May 1988, see entire document.	1-46

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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